Immunohistochemistry
Principles and Applications

Introduction

- Immunohistochemistry (IHC) combines histological, immunological and biochemical techniques for the identification of specific tissue components by means of a specific antigen/antibody reaction tagged with a visible label.

- IHC makes it possible to visualize the distribution and localization of specific cellular components within a cell or tissue.
Immunohistochemistry utilizes labeled antibodies to localize specific cell and tissue antigens, and is among the most sensitive and specific histochemical techniques.
▪ 1945 – Albert Coons 1st used an Ab labeled with a fluorescent dye to visualize tissues.

▪ 1966 – 1st developed enzyme labeling instead of fluorescent label.

▪ 1974 – IHC was performed for the 1st time on routine formalin fixed paraffin embedded sections.

▪ 1981 – developed avidin-biotin labeling

▪ 1991 – Heat induced antigen retrieval technique in IHC was done

▪ 1995 – Polymer technology introduced
**TERMINOLOGIES**

- **ANTIGENS** - Molecules that induces formation of an Ab and is foreign to the animal into which it is introduced

- Sites on Ag that are capable of inducing Ab formation are known as – **EPITOPES/ ANTIGENIC DETERMINANT** – the exact site on the Ag with which the Ab combines

- **ANTIBODIES** – IgG is the most frequently used Ab for IHC
  - The **paratope** of Ab binds to the epitope of Ag
  - Abs are also proteins - thus any part of the Ab may itself serve as epitope to induce Ab formation (to which secondary Ab binds)
  - IHC technique prove that Ig molecules can serve both as Ab and Ag
IHC CAN BE PERFORMED ON:

- Formalin fixed paraffin embedded sections
- Frozen sections
- Smears
- Imprints
- Cytospins

METHODS:

DIRECT
- One step staining method
- Labeled Ab reacts directly with Ag in tissue

INDIRECT
- Unlabeled primary Ab reacts with tissue Ag
- Conjugated second Ab reacts against primary Ab
1. Peroxidase-antiperoxidase method (PAP)
2. Biotin-avidin complex method (ABC)
3. Labeled streptavidin-biotin method (LSAB)
4. Alkaline phosphatase- anti alkaline phosphatase methos (APAAP)
5. Polymer based labeling

Direct Method

It has the advantages of rapidity, ease of performance and limited nonspecific reaction.
Indirect Method - Procedure

An unlabeled primary antibody binds to the tissue antigen.

Two-Step Indirect Method

An enzyme-labeled secondary antibody binds to the primary antibody.
Multiple Immunolabeling

IHC PROTOCOL

Fixation and processing

Section cutting

Deparaffinisation and rehydration
Blocking endogenous peroxidase

Blocking non-specific antibody binding

Antigen retrieval

Primary antibody

Secondary antibody

Chromogen

Chromogen enhancement

Counterstain

Stringent washing between reagents

Mount
Avidin-Biotin Methods

• Uses the strong and high affinity of avidin (egg white glycoprotein) for biotin (water-soluble vitamin).

• Avidin has four binding sites for biotin but fewer than four molecules of biotin will actually bind to avidin.

Avidin-Biotin Methods

• Two of the most common methods include
  • Avidin-Biotin enzyme Complex (ABC)
  • Labeled StreptAvidin-Biotin (LSAB)
ABC Method

• The enzyme complex is prepared by mixing biotinylated enzyme (HRP or AP) and avidin.

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biotinylated enzyme + avidin = avidin-biotin-enzyme complex
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- This preformed avidin-biotin-enzyme complex then reacts with the biotinylated secondary antibody.

ABC - Procedure

An unlabeled primary antibody binds to the antigen.

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antigen primary Ab (mouse)
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A biotinylated secondary antibody binds to the primary antibody.

A preformed avidin-biotin-enzyme complex solution is added and binds to the biotinylated secondary antibody.
ABC - Procedure

A substrate-chromogen solution is added ending the reaction and producing a colored end-product.

LSAB Method

• Uses enzyme-conjugated streptavidin. Streptavidin is conjugated to several molecules of enzyme horseradish peroxidase (HRP) or alkaline phosphatase (AP).

• The secondary antibody is conjugated to numerous biotin molecules, each of which can potentially bind to an enzyme-conjugated streptavidin.
LSAB – Procedure

An unlabeled primary antibody binds to tissue antigen.

A biotinylated secondary antibody binds to the primary antibody.

Each secondary antibody contains multiple biotin molecules; several secondary antibodies can bind to the primary antibody.
LSAB – Procedure

An enzyme-labeled streptavidin is added and binds to the secondary antibody.

LSAB – Procedure

A substrate-chromogen solution is added producing a colored end-product.
Mc used labels in IHC are enzymes

Enzymes used are –

- Horseradish Peroxidase (HRP)
- Alkaline Phosphatase (Calf Intestinal)
- Glucose Oxidase
- β-D Galactosidase (Bacterial Derived)
AEC and DAB

Examples of staining results using AEC and DAB chromogens.
Polyclonal Antibodies

Polyclonal antibodies reacting with various epitopes
Each antibody is made by a different B-cell

Monoclonal Antibodies

Monoclonal antibodies reacting with similar epitopes
APPLICATION OF IHC IN ROUTINE SETTINGS

DIAGNOSIS OF TUMORS

PROGNOSTIC MARKER

PREDICTIVE OR THERANOSTIC MARKERS

IDENTIFICATION OF INFECTIOUS ORGANISMS

1. Maximum utility of IHC is in distinguishing carcinoma from lymphoma, sarcoma and melanoma
2. Workup of hematolymphoid neoplasms
3. Metastatic carcinoma of unknown primary (CUP)
4. Soft tissue neoplasms – 4 common diagnostic setting
   a. Small round cell tumors
   b. Monomorphic spindle cell tumors
   c. Epithelioid soft tissue tumors
   d. Pleomorphic spindle cell tumors
5. In bone – to differentiate primary from metastatic non–osseous tumors
6. CNS tumors
7. Germ cell tumors

PROGNOSTIC MARKERS

1. Loss of myoepithelial or basal cells or basement membrane/collagen type IV – these allow assessment of microinvasion
2. Endothelial markers – assist in identification of lymphovascular spaces to ascertain tumor embolism
3. ER, PR and her2/neu
4. Ki-67 /MIB-1 – proliferation markers
**PREDICTIVE OR THERANOSTIC MARKERS**

1. ER/PR – tamoxifen in Ca. breast
2. Her 2 – herceptin in breast cancer
3. C-kit – gleevec/imatinib in GIST, CML
4. CD20 – rituximab in B-cell NHL
5. EGFR – erlotinib in lung cancer

**IDENTIFICATION OF INFECTIOUS ORGANISMS**

1. Viruses – HSV, CMV, EBV
2. Others – toxoplasma, pneumocystis